Angiographic Effects of Indocyanine Green Photobleaching by the Diode Laser

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■ BACKGROUND AND OBJECTIVE: To investigate the cause of hypofluorescent spots detected by indocyanine green (ICG) videoangiography in areas subjected to ICG-enhanced transpupillary thermotherapy in pigmented rabbits.

■ MATERIALS AND METHODS: In 6 eyes, two similar areas were treated with transpupillary thermotherapy. A standard dose of ICG (0.5 mg/kg) was injected intravenously before treatment of the second area. Red-free photographs without further injection of ICG (first ICG videoangiography) were then performed. The first area was re-irradiated using the same parameters. Red-free photographs and a second ICG videoangiography, still without further injection of ICG, were performed. ICG was then re-injected and a third ICG videoangiography was obtained. Finally, fluorescein angiography was performed.

■ RESULTS: The first ICG videoangiography demonstrated hyperfluorescence of the first area and normal fluorescence of the second area. The second ICG videoangiography demonstrated hypofluorescence of the first area. The third ICG videoangiography showed hyperfluorescence of both areas.

■ CONCLUSIONS: Hypofluorescence detected after re-irradiation is probably related to ICG photobleaching.

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INTRODUCTION

Age-related macular degeneration is, in several industrialized countries, the leading cause of central visual loss in patients older than 65 years.1 The exudative form of the disease, characterized by the development of choroidal neovascularization, represents approximately 80% to 90% of cases of legal blindness attributed to the disease.2,3 Transpupillary thermotherapy (TTT)4 and TTT enhanced by intravenous injection of standard5 and higher doses of indocyanine green (ICG)6,7 are promising treatment possibilities.

The use of ICG as an adjunct to retinal irradiation for treatment of choroidal neovascularization can induce a hypofluorescent spot on ICG videoangiography performed, without dye re-injection, immediately after treatment.8 Hypofluorescent spots were also observed
in three patients who received TTT or diode laser photocoagulation after undergoing ICG videoangiography for diagnostic purposes.9

Previous studies demonstrated that some photosensitizers may lose their photochemical and fluorescent properties when exposed to light10-13 and reported accentuated ICG photobleaching in vitro after intense diode laser exposure (> 1,000 J/cm²) at a wavelength of 805 nm.14 The current experimental study examined whether photobleaching of ICG with a diode laser could contribute to the occurrence of hypofluorescent spots on ICG videoangiography performed without re-injection of the dye after ICG-enhanced TTT in pigmented rabbits.

**MATERIALS AND METHODS**

Six eyes of six pigmented rabbits were used in the current experimental study. To perform TTT, a standard Mainster OMRA-S contact lens (Ocular Instruments, Bellevue, WA) and a modified 810-nm diode laser (Iris Medical Instruments, Mountain View, CA) coupled to a slit-lamp biomicroscope (Topcon Co., Walnut Creek, CA) were used. The sequence of events regarding the experimental design is schematized in Figure 1.

Without prior intravenous ICG injection, a well-defined photocoagulation lesion was produced in the inferior retina of each eye using a 3-mm diameter spot, 400 mW power, and the exposure time required for the appearance of a white chorioretinal lesion with distinct borders. Three minutes after intravenous injection of ICG (0.5 mg/kg), a second photocoagulation lesion was produced using the same laser parameters.

Immediately after treatment, red-free photographs and ICG videoangiography without further dye injection (first ICG videoangiography) were performed. The first area, which was irradiated prior to dye injection, was then re-irradiated using the same laser parameters, with a 10-second exposure time. Red-free photographs and ICG videoangiography were performed again immediately after, without dye re-injection (second ICG videoangiography). The same dose of ICG and sodium fluorescein at 10% in a 0.1 mg/kg dose were then injected prior to performing fluorescein angiography and a third ICG videoangiography.

**RESULTS**

Red-free photographs performed immediately after TTT revealed similar well-defined white lesions (Fig. 2A).

ICG videoangiography performed without dye re-injection (first ICG videoangiography) presented less similar findings (Fig. 2B). In all six eyes, the lesion produced before ICG administration had marked hyperfluorescence. In the lesions produced after dye administration, two were normofluorescent (Fig. 2B), three were slightly hyperfluorescent with a hypofluorescent border, and one was hypofluorescent.

After re-irradiation of the first area, previously treated prior to ICG administration, red-free photographs revealed insignificant alterations of the lesions, such as minor additional whitening and a slight increase in area (Fig. 2C). ICG videoangiography performed without dye re-injection (second ICG videoangiography) revealed marked hypofluorescence, corresponding exactly to the area that was re-irradiated, and complete disappearance of the previously detected

First photocoagulation lesion

−

Intravenous injection of indocyanine green (ICG)

−

Second photocoagulation lesion

−

Red-free photographs and ICG videoangiography without dye re-injection

(First lesion: hyperfluorescent/Second lesion: normo-fluorescent)

−

Re-irradiation of the first lesion

−

Red-free photographs and ICG videoangiography without dye re-injection

(First lesion: hypofluorescent/Second lesion: normo-fluorescent)

−

Intravenous injection of ICG and sodium fluorescein at 10%

−

ICG videoangiography and fluorescein angiography

(Both lesions: hyperfluorescent)

Figure 1. Sequence of events regarding the experimental design.
Figure 2. (A) Red-free photograph of the lesions produced before (area 1) and after (area 2) intravenous injection of indocyanine green (ICG). (B) ICG videoangiography performed, without further injection of ICG, soon after transpupillary thermotherapy. Hyperfluorescence in area 1 and normofluorescence in area 2. (C) A red-free photograph produced after re-irradiation of area 1, showing additional whitening of this area. (D) ICG videoangiography performed, without further injection of ICG, after re-irradiation of area 1, showing marked hypofluorescence corresponding to the re-irradiated area. Area 2 remains unchanged. (E) ICG videoangiography performed after intravenous re-injection of ICG. Hyperfluorescence in both areas. (F) Fluorescein angiography performed after re-irradiation of area 1, showing hyperfluorescent lesions.
hyperfluorescent lesion (Fig. 2D). ICG videoangiography performed after dye re-injection (third ICG videoangiography) demonstrated increased similar hyperfluorescence of both lesions in all six eyes (Fig. 2E).

In all 12 lesioned areas, fluorescein angiography revealed marked hyperfluorescence of the lesion borders (Fig. 2F). The center of the treated area was hypofluorescent, normofluorescent, or hyperfluorescent.

**DISCUSSION**

We previously reported that ICG videoangiography performed without dye re-injection in 12 of 12 eyes subjected to TTT enhanced by intravenous injection of ICG (25 mg) revealed hypofluorescent spots. In 8 of 23 areas treated with ICG-enhanced TTT, hypofluorescent spots on the ICG videoangiography performed without dye re-injection were observed. In all eight areas, the hypofluorescent spots disappeared on the ICG videoangiography performed after intravenous administration of the same dose of ICG. None of the 25 areas treated with TTT alone had such hypofluorescent spots.

Previous studies demonstrated that several known photosensitizers lose their photochemical and fluorescent properties when exposed to light. Holzer et al. demonstrated ICG thermoinstability and photoinstability in an experiment performed in vitro, and reported the important stabilizing role of the binding of ICG to plasma proteins with a high molecular weight. Varriale et al. reported strong ICG photobleaching in vitro after intense 805-nm diode laser exposure (> 1,000 J/cm²). Mang et al., studying the photobleaching of porphyrins used in photodynamic therapy of experimental tumors, stated it is likely that the porphyrin that undergoes photobleaching is the same as that which is responsible for photodynamic destruction of the tumor.

In the current study, the first ICG videoangiography demonstrated important ICG molecule aggregation in the chorioretinal lesion that was produced by irradiation prior to ICG injection. This finding was consistent with previous descriptions of hyperfluorescent ICG videoangiography lesions in choroidal, retinal, and scleral diseases associated with disturbances of the internal and external hemato-retainal barriers.

Because there was a chorioretinal lesion of the same intensity produced in an area irradiated after ICG administration, it is likely that this area was also impregnated with ICG. However, accentuated hyperfluorescence was absent in all of the lesions. In those areas, it is possible that fluorescence of the molecules that gradually impregnated the chorio-retinal lesion was inactivated (photobleached) due to the retinal irradiation. The result was a consistently less intense fluorescence than that in the first irradiated area. This theory is reinforced by the fact that a new intravenous injection of ICG rendered the second lesion area hyperfluorescent (third ICG videoangiography). However, it is important to note that, due to the short half-life of ICG (estimated to be 2 to 4 minutes), the low blood dye concentration at the time of the second area irradiation, initiated 3 minutes after ICG administration, could have contributed to this finding.

In the area that was re-irradiated, the ICG videoangiography performed prior to dye re-injection (second ICG videoangiography) demonstrated that the lesion completely disappeared, possibly through the same mechanism—photobleaching of the irradiated ICG molecules. This is why dye re-injection induced the reappearance of the lesion (third ICG videoangiography), although with variable fluorescence patterns. However, such variations can be explained by the re-irradiation of the first treated area, especially if one considers the high doses of irradiation used in this experiment, which probably caused significant changes in the histopathologic organization of the retinal and choroidal tissues.

Previous studies in humans demonstrated a hyperfluorescent spot in 100% of the areas submitted to diode laser treatment performed several minutes after intravenous ICG injection. Interestingly, in the current study, all areas irradiated 3 minutes after ICG administration were normofluorescent. It was estimated that ICG has a half-life of 2 to 4 minutes. It is then probable that binding of circulating ICG to the lesion may have contributed to the lack of hypofluorescence in these areas.
In the previous studies,\textsuperscript{8,9} there was no circulating ICG during the treatment because irradiation was performed only several minutes after ICG administration, which caused the hypofluorescent spot to be detected in all treated areas. Late aggregation of circulating ICG could also explain the absence of a hypofluorescent spot in 15 of the 23 areas treated 30 seconds after intravenous ICG injection in our previous study.\textsuperscript{15}

The current study demonstrated angiographically the occurrence of ICG photobleaching, which seems to have been responsible for the observation of hypofluorescent spots previously detected in humans,\textsuperscript{8,9} as well as in experiments on subthreshold TTT enhanced by standard doses of ICG in pigmented rabbits.\textsuperscript{15} In fact, the findings of the current study indicate that, before performing ICG videoangiography, a new administration of ICG in eyes treated with ICG-enhanced TTT is necessary so that one can correctly study the choroidal effects of the treatment. It was also demonstrated that circulating ICG exerts an important effect on ICG videoangiography in areas irradiated soon after ICG administration. The possible repercussions of the observed photobleaching on the eventual photodynamic effect of ICG are not clear.

REFERENCES